Effect of Growth Temperature on Chloroplast Structure and Activity in Barley

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ABSTRACT

Seedlings of barley (*Hordeum vulgare* L. cv. Abyssinian) were grown at constant temperature and light intensity and the properties and structure of chloroplasts in the primary leaf were examined. Seventeen growth temperatures ranging from 2 to 37 C were employed. Three major effects of the growth temperature were seen. (a) At very low and high growth temperatures chloroplast biogenesis was inhibited. This occurred in plants grown at temperatures above 32 C while growth at 2 C resulted in a mixed population of pale yellow, pale green, and green plants. (b) Chloroplasts were produced at all other temperatures tested but growth temperatures within a few degrees of those inhibitory to chloroplast development resulted in chloroplasts with abnormal properties and structure. Chloroplasts in the green plants grown at 2 and 5 C showed a number of structural peculiarities, including a characteristic crimping of granal thylakoids. Photoreductive activity, measured using ferricyanide as the Hill oxidant in the presence of gramicidin D, was high, but this activity in chloroplasts isolated from plants grown at 2 C showed thermal inactivation at temperatures 5 degrees lower than was the case with plants grown at higher temperatures. High growth temperatures (30 to 32 C) yielded chloroplasts with reduced photoreductive activity and a tendency toward the formation of large grana and disorientation of the lamellar systems with respect to one another. Chloroplasts of the most affected plants (grown at 32 C) frequently contained a very large elongated granum, with narrow intrathylakoid spaces. (c) Photoreductive activity was not constant at intermediate growth temperatures but steadily declined with decreasing growth temperatures between 27 and 11 C. Some alterations in chloroplast structure were also observed.

The changes in chloroplast activity and structure indicate that acclimation to temperature takes place over the entire temperature range in which chloroplast development is permitted.

Temperature, light, and other environmental factors can affect the course of chloroplast development in higher plants and algae. High temperatures (above 32 C) inhibit the production of normal chloroplasts in *Euglena* (18) and in higher plants (9), while low temperatures (10–16 C) can inhibit chloroplast development in chilling-sensitive plants such as sugarcane (7), sorghum (20), and maize (13). Temperatures intermediate between these extremes may also affect differentiation of the photosynthetic apparatus. Aside from considerations of genotypic adaptation to temperature, many plants show an ability to acclimate to the prevailing temperature regime during their growth. The rate and temperature optimum of photosynthesis in leaves and the leaf anatomy can vary according to the growth temperature and light intensity (2, 3, 11, 21). Little is known about changes in the photosynthetic electron transfer system of chloroplast membranes as the result of acclimation to temperature. To study this aspect, as well as the effect of growth temperature on chloroplast differentiation, barley

seedlings were grown at temperatures ranging from 2 to 36 C under constant light intensity. The plants were then examined for differences in chloroplast structure and activity.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Seeds of barley (*Hordeum vulgare* cv. Abyssinian) were washed in running tap water for 24 hr and then planted in Vermiculite, previously soaked in Hoagland solution and drained, in weighted glass tubes (4 × 30 cm). The top of each tube was covered with Gladwrap film (Union Carbide) which allows gas exchange while reducing water loss and immersed to within 1 to 2 cm of the top of the tube in water contained in an insulated water bath. The baths were maintained at temperatures from 11 to 37 C (± 0.10 C) and continuous overhead illumination of 4,500 lux at seed level was provided by white fluorescent lights. The plants were harvested when they had reached a height of 17 to 20 cm (6–21 days depending on the temperature). Plants grown at 2 and 5 C were raised under similar conditions except that temperature-controlled rooms were used instead of water baths.

Chloroplast Isolation and Assay. Chloroplast thylakoids were isolated from 1 to 5 g fresh wt of leaf tissue as previously described (16). The top half of the primary leaf less 1 cm of the leaf tip was used.

The photoreduction of ferricyanide was assayed spectrophotometrically in a reaction mixture (1.5 ml) containing chloroplasts (4 μg of Chl ml^{-1}), 50 mM Sørensen's phosphate buffer (pH 7.5), 50 mM NaCl, 0.05% (w/v) BSA, gramicidin D (4 μg ml^{-1}), and 0.34 mM K-ferricyanide. Actinic light (11 × 10^4 ergs cm^{-2} sec^{-1}) filtered through a Corning 2-60 red cut-off filter was supplied from a 150-w tungsten-halogen lamp.

Chl concentrations in leaves and chloroplast suspensions were determined using the procedure described by Arnon (1).

Electron Microscopy. The primary leaf tissue between 3 to 4 cm below the tip was cut into pieces (3 × 0.5 mm) and fixed at 20 C in formaldehyde-glutaraldehyde (12) containing 7% sucrose. After washing in 0.1 M phosphate buffer (pH 7.2) containing 7% sucrose, the tissue was postfixed for 2 hr in 1% OsO$_4$, stained in 2% aqueous uranyl acetate for 30 min, washed and dehydrated through a graded series of alcohol, and embedded in Epon 812. Thin sections were stained with 2% uranyl acetate and with lead citrate and examined in a Siemens Elmskop I electron microscope.

RESULTS

Inhibition of Chloroplast Development by High and Low Growth Temperatures. To study the effect of high temperatures on seedling growth and chloroplast development, barley was grown at temperatures ranging from 29 to 37 C at 1-degree intervals. Growth rates indicated by the rate of elongation of the primary leaf decreased gradually with increasing temperature to 34 C

where the rate was about 40% of that at 29 C. The growth had declined to 10 to 20% at 35 C and plants at 36 and 37 C died after making some slow growth for 2 to 3 days. The total Chl content of the primary leaf (less the top 1 cm) showed little change at growth temperatures of 29 to 31 C but decreased by 50 to 75% at 32 C. Only traces of Chl (less than 2% of the 29 C value) were detected in leaves of plants grown at 33 and 34 C and none in plants grown at 35 C. Thus, the cut-off for Chl synthesis was sharp, occurring over a temperature span of less than 2 centigrade degrees, and it took place at about 3 degrees lower than the upper temperature limit for growth. High temperature repression of chloroplast development of the type seen in barley at 33 C occurs in several cereals and has been studied extensively by Feierabend (9, 10, 19).

Low temperatures just above freezing can also result in inhibition of chloroplast development in barley even though the seedling leaves continue to grow, albeit slowly. When barley was grown at 2 C under constant illumination 5.8% of the plants were pale yellow, yellow-green, or pale green (Fig. 1). The rest of the plants in the population were green. In contrast, all plants were green in a population of 1,000 grown at 5 C. Chloroplast development was never completely repressed at 2 C and even plants with pale yellow leaves contained traces of Chl (Fig. 1). When these or the yellow-green plants were transferred to 22 C in light the plants grew rapidly, doubling in height in 24 hr. There was a lasting effect of the low temperature on Chl development in that part of the leaf which had elongated at 2 C. It turned pale green but never became green while the bottom half of the leaf which had elongated at 22 C was green. Upon transfer to 22 C the pale-green-leaved plants turned green after 3 days.

A temperature of 2 C appears to be a borderline temperature for chloroplast development in barley and this probably accounts for the variation within the population. The variation in and extent of chloroplast repression at 2 C are shown in Figure 2. Figure 2A shows a plastid in a pale yellow leaf and Figure 2B a plastid in a pale green leaf. The majority of cells in the pale yellow leaves contained plastids with vesicles but hardly any recognizable thylakoids, whereas those from the pale green leaves contained

stromal thylakoids and small grana consisting most commonly of two or three thylakoids.

Occasionally cells were found in the pale yellow leaves that contained chloroplasts with well defined thylakoids of the type shown in Figure 2B, and this would account for the small amounts of Chl found in these leaves (Fig. 1). The pale green leaves contained a few cells in which all of the plastids were of the type shown in Figure 2A.

A characteristic feature of plastids of all plants grown at 2 C were regions of low electron density. These may have been protein bodies that are often found in plastids of plants undergoing some form of stress. It is not clear if DNA fibrils were present. These bodies were not found in barley grown at 5 C, or at higher temperatures. Osmiophilic bodies were also prominent in plants grown at 2 C.

Development of Abnormal Chloroplasts at Low and High Growth Temperatures. Figure 2C shows thylakoid arrangements found in chloroplasts in green leaves of barley grown at 2 C. Stromal and granal thylakoids were present. Most of the grana contained four to seven thylakoids, a considerably smaller number than in plants grown at warmer temperatures (see below). A notable feature of the majority of chloroplasts was a conspicuous crimping of membranes. This was particularly noticeable in granal thyakoids but also occurred in stromal thylakoids and the plastid envelope. It was also seen in chloroplasts of plants grown at 5 C, but not in plants grown at 11 C and at higher temperatures. There was also considerable variation in the width of intrathylakoid spaces, some of which were very narrow (Fig. 2C), while in other cases the thylakoids appeared to be swollen (Fig. 2D). Protein bodies were still common in these chloroplasts but osmiophilic bodies were rare.

The photoreductive activity of the thylakoids isolated from the green plants grown at 2 C was very high (see below). However, this activity was considerably more heat-labile than in chloroplasts isolated from plants grown at higher temperatures. Photoreductive activity became inactivated at temperatures 5 degrees lower than in thylakoids isolated from plants grown at 27 C (Fig. 3).

The chloroplasts produced in plants grown at high temperatures were very different in appearance from those grown at cold temperatures. In the leaf cells of plants grown at 31 C and in some plants grown at 32 C, large grana were common (Fig. 4A). The grana were more disoriented with respect to each other and thylakoids interconnecting grana were fewer in number than in chloroplasts of plants grown between 11 and 27 C. In these respects they resembled the "grana-rich" chloroplasts of barley mutants described by Nielsen *et al.* (15). Many of the chloroplasts in plants grown at 32 C showed a much more severe effect of the high growth temperature. Much of the membranous material was condensed into a large strap-like granum (Fig. 4B). There were also a number of apparently randomly distributed grana, small in diameter and usually of no more than two or three thylakoids. Some of these, like the large elongated grana, were intensely stained.

The photoreductive activity of chloroplasts isolated from barley grown at 32 C was less than half that of chloroplasts from plants grown at 27 C, and it was slightly more heat-labile (Fig. 3). Thus, both cold and heat stresses decrease the thermal stability of the photosynthetic apparatus in barley and a temperature-induced enhancement of thermal stability similar to that which occurs in *Atriplex* (17) was not seen.

Chloroplast Activity and Structure at Moderate Growth Temperatures. Figure 5 shows the photoreductive activity measured at 24 C of chloroplasts isolated from plants grown at different growth temperatures. For growth temperatures above 11 C, maximal activity was found with chloroplasts isolated from plants grown at 27 to 28 C. At higher temperatures, not unexpectedly, the activity declined as the growth temperature was increased. Less expected was a steady decline in activity with decreasing growth tempera-

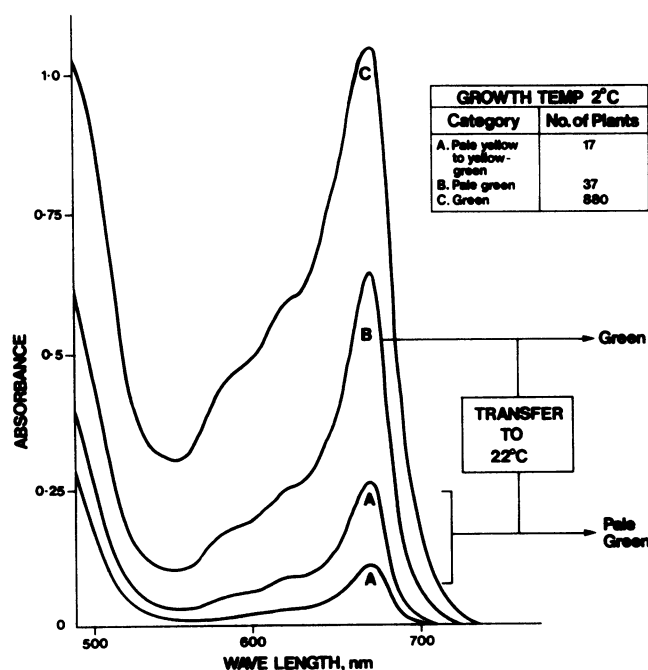| GROWTH TEMP 2°C	
Category	**No. of Plants**
A. Pale yellow to yellow-green	17
B. Pale green	37
C. Green	880

FIG. 1. Absorption spectrum of primary leaf of barley germinated and grown at 2 C in continuous light. Leaves were harvested 69 days after germination (average height 28 mm). Plants were divided into three categories: A, pale yellow or yellow-green; B, pale green; C, green.
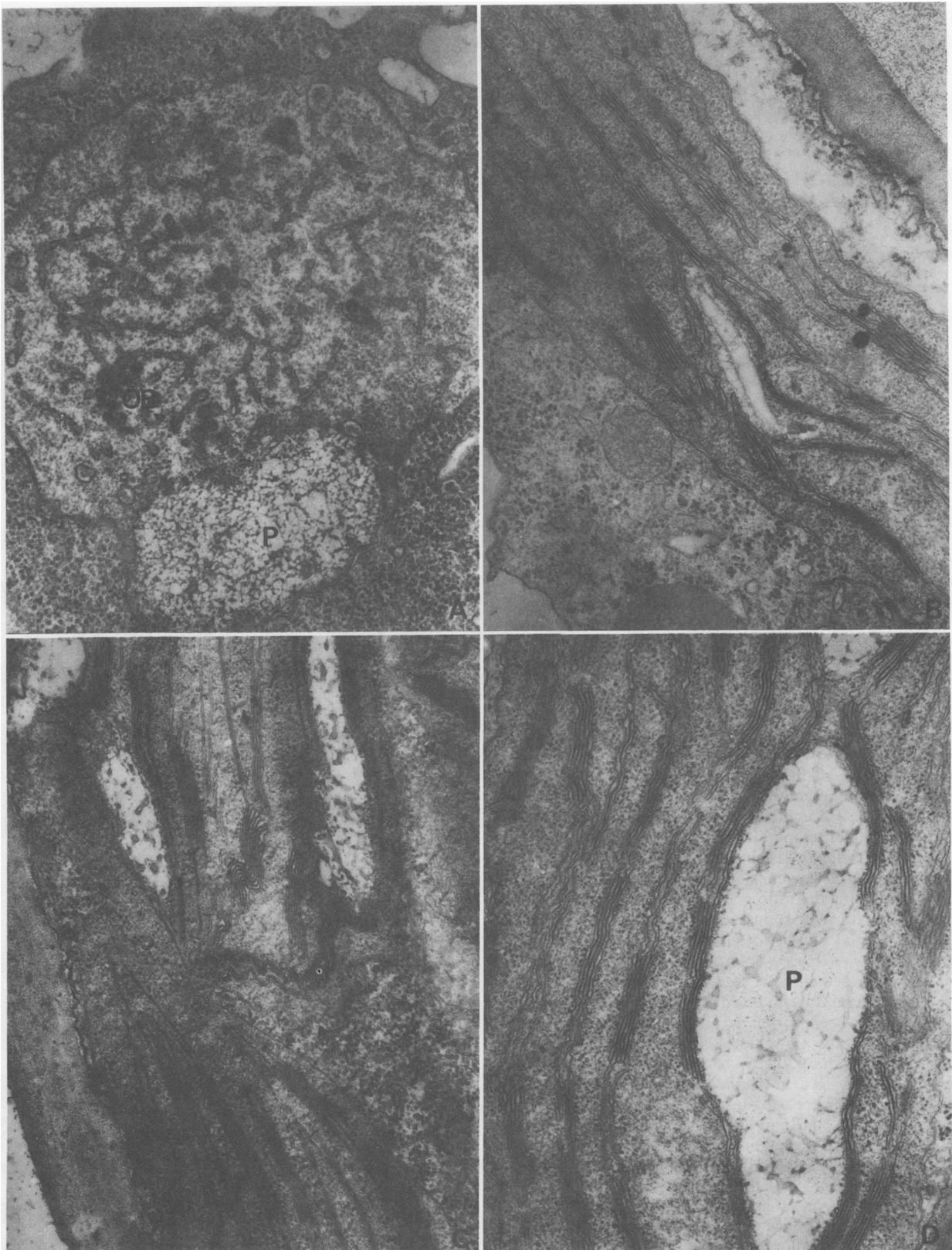
FIG. 2. Electron micrographs of plastids from barley grown at 2 C (× 30,000). A: plastid in a pale yellow leaf (category A, Fig. 1) showing vesicles, osmiophilic bodies (OB), and a protein body (P). Plastid ribosomes appear to be present but are reduced in numbers compared with plastids containing thylakoids (cf. Fig. 2D). B: chloroplast in a pale green leaf (category B, Fig. 1) showing stromal thylakoids and grana made up of a few thylakoids. C: chloroplasts in a green leaf (category C, Fig. 1) showing exaggerated crimping of the granal thylakoids and grana more normal in appearance but having very narrow intrathyalkoid spaces. The plastid envelope is also frequently crimped in plants grown at 2 C (▲). D: detail showing a protein body (P), and crimped stromal and granal thylakoids characteristic of plants grown at 2 and 5 C (material from a green leaf, category C, Fig. 1).
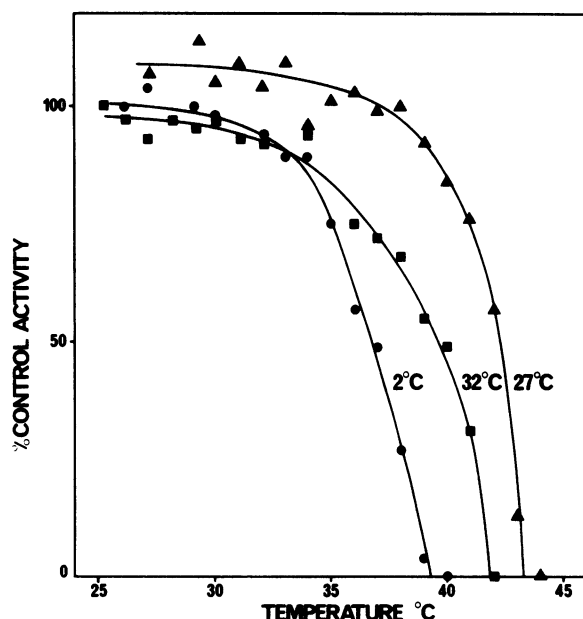
FIG. 3. Heat lability of thylakoids isolated from plants grown at 2, 27, and 32 C. The isolated thylakoids were heated to various temperatures and then assayed for photoreduction of ferricyanide at 24 C as follows. Aliquots (20 μl) of thylakoid suspension (300 mg of Chl ml^{-1}) were added to cuvettes immersed in a water bath maintained at temperatures ranging from 25 to 44 C as indicated on the x axis of the figure. After 4 min the cuvette was transferred to a water bath at 24 C and reaction mixture (1.5 ml at 24 C) was added. After a further 2 min for temperature equilibration, photoreductive activity was assayed at 24 C. The results are plotted as a percentage of the activity in control samples which were not heated (rates were 685, 579, and 238 μmol of ferricyanide reduced hr^{-1} [mg of Chl]$^{-1}$ for plants grown at 2, 27, and 32 C, respectively). (●——●): plants grown at 2 C; (▲——▲): plants grown at 27 C; (■——■): plants grown at 32 C.

tures between 27 and 11 C.

Highest activity on a Chl basis was obtained with plants grown at the two lowest temperatures. These plants were very much stunted, severely at 2 C and less so at 5 C, when compared with plants grown at higher temperatures.

The ratio of activity in the presence of gramicidin D to the activity in its absence was constant at all growth temperatures. This was also true for the ratio of Chl a to Chl b in the isolated chloroplasts. Growth temperatures between 5 and 31 C also did not affect the thermal stability of Hill activity.

The Hill activity of barley chloroplasts shows an increase in apparent Arrhenius activation energy below 20 C and again below 9 C (16). These temperature-induced changes in chloroplast membrane activity have been correlated with changes in Q_{10} around the same temperatures for chloroplast development and leaf growth (22). Rubidium uptake by barley root tips also shows an increase in Q_{10} below 10 C in plants grown at 28 C but not when plants grown at 10 C were used instead (5). Figure 6 shows that changes in slope near 20 C and 9 C in an Arrhenius plot for Hill reaction activity are still apparent in the chloroplasts with high photoreductive activity isolated from plants grown at 2 C as well as in the less active ones isolated from plants grown at 11 C.

Since the chloroplasts isolated from plants grown at 11 and 27 C displayed differences in Hill activity, it is conceivable that there were also differences in chloroplast structure. An analysis of the thylakoid structure from electron micrographs of chloroplasts in the primary leaf of plants grown at the two temperatures is given in Table I. Chloroplasts of plants grown at 11 C had a higher ratio of granal thylakoids to stromal thylakoids than in plants grown at 27 C. The average dimensions of grana were similar although the average width of grana and thickness of the granal thylakoids were slightly higher in the case of the plants grown at 27 C. The frequency of occurrence of different sized grana also revealed a

FIG. 4. Electron micrographs of chloroplasts in barley grown at 32 C (× 30,000). A: chloroplast of a type seen in some cells of plants grown at 32 C and in most cells of plants grown at 31 C. These chloroplasts contain large multilamellar grana more disoriented with respect to one another than grana from chloroplasts in plants grown at lower temperatures. Stromal thylakoids appear to be reduced in number and some are swollen. B: chloroplast typical of those in plants grown at 32 C showing numerous small grana and an intensely stained elongated granum with narrow intrathylakoid spaces. Osmiophilic bodies are present in both types of chloroplast.
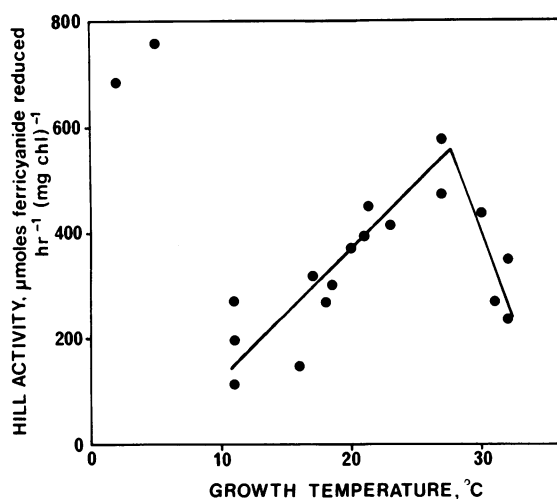
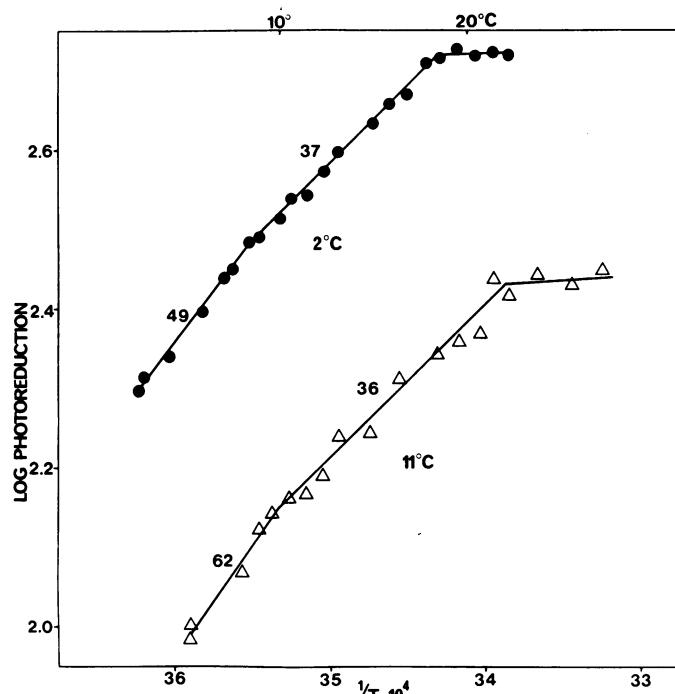FIG. 5. Hill activity (measured at 24 C) of chloroplasts isolated from barley grown at different temperatures.

FIG. 6. Arrhenius plot of the photoreductive activity in μmol of ferricyanide photoreduced hr^{-1} (mg of Chl)$^{-1}$ of chloroplasts isolated from barley grown at 2 and 11 C.

tendency toward grana with a large number of thylakoids (12–50) in chloroplasts of the plants grown at 27 C compared with those grown at 11 C. The plants grown at 27 C also contained a greater number of small grana consisting of two appressed thylakoids.

DISCUSSION

It has been customary to study phenotypic acclimation by plants to the temperature of the environment by comparing plants grown under several different regimes of day and night temperatures in growth chambers. A different approach has been adopted here in that the influence of growth temperature on the differentiation of chloroplasts in barley seedlings was studied by growing the plants under conditions of high humidity, constant temperature, and constant light intensity. While these growth conditions are far from those existing in the field, their choice nonetheless was

deliberate. It was reasoned that specific effects of temperature on chloroplast development would be more readily apparent if other environmental variables were eliminated as far as possible. Also by using growth vessels immersed in water baths it becomes feasible to study the effects of a large number of growth temperatures at the one time.

The development of chlorotic leaves is a common symptom in plants subjected to stress. Chlorosis can result from mineral deficiency, treatment with certain herbicides, fungal infection, and also elevated temperatures (9). Low temperatures can inhibit Chl synthesis (6, 13, 14, 24) and in sugarcane (7) and sorghum (20), chlorotic bands may appear following a series of low night temperatures. In cucumber leaves the combined effects of light and cold lead to rapid loss of Chl (25).

In rye seedlings grown at 32 C the synthesis of chloroplast ribosomes and fraction I protein as well as that of Chl and carotenoids is inhibited, but cytoplasmic ribosomes and several enzymes of the Calvin cycle are made (10). It is not known whether inhibition of chloroplast development in barley at 2 C (Fig. 1) also involves an inhibition of plastid ribosomal RNA synthesis. However, Slack et al. (20) have demonstrated an inhibition of the synthesis of chloroplast ribosomal RNA in sorghum mesophyll chloroplasts following a single cold night. Consistent with this observation is the apparent paucity of plastid ribosomes in the pale yellow barley compared with green barley grown also at 2 C (Fig. 2). These results suggest that both the cold and hot treatments may result in a specific inhibition of synthesis of chloroplast DNA or its transcription during plastid replication.

Chloroplasts of plants grown at temperatures close to those inhibiting chloroplast biogenesis are active photochemically but show structural abnormalities. Grana apparently equivalent to the elongated granal species produced at high temperatures and the crimped granal thylakoids of the low-temperature-grown barley have been described in xantha and viridis mutants of barley grown at 21 C (23). This suggests that some of the alterations in the regulatory control for chloroplast thylakoid biogenesis brought about by nuclear gene mutations can be mimicked in the wild type plant subjected to temperature stresses. It is also of interest that the crimped granal thylakoids were seen only in the barley grown at 2 or 5 C which are "frost-hardening" temperatures. Exposure of many cereals to these low temperatures produces compositional changes in lipid such as an increased linolenic acid content (e.g. ref. 8). The mutants of barley having crimped thylakoids, the xantha-m group, show a higher than normal (compared with wild type) incorporation of acetate into monogalactosyl diglyceride and a preferential labeling of linolenic acid in this lipid (26).

In the more normal temperature range for growth, one striking effect of growth temperature is the increase in photoreductive activity with increasing growth temperatures between 11 and 27 C (Fig. 5). In contrast, changes in chloroplast structure between plants grown at 11 and 27 C were small (Table I). Since changes in the amount of antennae Chl are usually accompanied by

Table I.   Chloroplast Membrane Composition and Structure at Two Growth Temperatures

Plants were harvested at 21 days for growth at 11 C and 8 days for growth at 27 C and measurements made on the primary leaf.

	Growth temperature	
	11 C	27 C
Chlorophyll content of leaf (mg/g fresh wt)	1.07	1.04
Ratio of chlorophyll a to b	3.0	2.9
Ratio of grana to stroma	0.66	0.51
No. of thylakoids per granum	8.6$\pm$0.6	8.9$\pm$0.9
Av. width of granum (nm)	135$\pm$10	162$\pm$17
Width of granal thylakoid (nm)	15.7$\pm$0.36	18.2$\pm$0.42
Av. length of granum (nm)	324$\pm$24	310$\pm$30

structural changes especially in the size of the grana (4), it seems likely that the warmer temperatures promote synthesis of electron transfer components rather than synthesis of more antennae Chl.

From our studies, three levels of temperature effects on chloroplast development in barley can be discerned. At temperatures from 11 to 31 C there is acclimation to growth temperature in that photosynthetic electron transfer capacity and to a lesser extent chloroplast structure is altered depending upon the growth temperatures. Outside of this range both cold and very warm temperatures can result in the production of chloroplasts showing abnormal properties and structures. Finally, there are ranges of low and high temperature in which chloroplast biogenesis, but not other cellular growth processes, is inhibited.

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